1H MRS: A potential biomarker of in utero placental function

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List of abbreviations:

BMI – Body Mass Index

Choline/H_2O – The amplitude ratio of choline to water

EmCS – Emergency Caesarean Section

FGR– Fetal Growth Restriction

Glx – The combined spectral contribution for Glutamine and Glutamate

Glx/H_2O – The amplitude ratio of Glx to water

Lipid 1.3ppm/H_2O – The amplitude ratio of the lipid peak at 1.3ppm to water

Lipid 0.9ppm/H_2O – The amplitude ratio of the lipid peak at 0.9ppm to water

HFFD – Haag Ferguson Forceps Delivery

MVM – Microvillous plasma membrane

NNU – Neonatal Unit

RDS – Respiratory Distress

SGA – Small for Gestational Age

SVD – Spontaneous Vertex Delivery

Abstract

The placenta is a temporary organ that is essential for a healthy pregnancy. It performs several important functions including the transport of nutrients, the removal of waste products and the metabolism of certain substances. Placenta disorders have been found to account for over 50% of stillbirths. Despite this there are currently no methods available to directly and non-invasively assess placenta function *in utero*. The primary aim of this pilot study is to investigate the use of ^{1}H Magnetic Resonance Spectroscopy (MRS) for this purpose.

^{1}H MRS offers the possibility to detect several placental metabolites including choline, lipids and the amino acids glutamine and glutamate (Glx) that are vital to fetal development and placental function. Here *in utero* placenta spectra were acquired from 9 small for gestational age (SGA) pregnancies, a cohort who are at increased risk of perinatal morbidity and mortality, and also from 9 healthy gestation matched pregnancies. All subjects were between 26 and 39 weeks gestation. Placenta Glx, choline and lipids at 1.3 and 0.9ppm were quantified as amplitude ratios to that of intrinsic H_2O. Wilcoxon Signed Rank tests indicated a significant difference in Glx/H_2O ($p = 0.024$) between the two groups but not in choline/H_2O ($p=0.722$) or in either lipid/H_2O ratio (1.3ppm: $p= 0.813$, 0.9ppm: $p=0.058$).

This study has demonstrated that ^{1}H MRS has potential for detecting placental metabolites *in utero*. This warrants further investigation as a tool for monitoring placental function.

Introduction

Disorders of the placenta are associated with over 50% of stillbirths (1). Despite this there are currently no methods available to non-invasively and directly assess placenta function *in utero*. Most information about this temporary yet crucially important organ has been gathered from post-delivery assessments, an exercise that has been likened to a post-mortem examination (2). There is an urgent clinical need to be able to monitor the function of the living placenta, as it supports the developing fetus, both to improve our understanding of this organ and to enable the effective identification and management of the at-risk fetus.

There is potential value in the direct detection of placental choline, lipids and the amino acids glutamine and glutamate as they are all vital to placental metabolism and fetal development. These metabolites can be detected using ^{1}H MRS but its application to the *in utero* placenta is novel. The primary aim of this pilot study is to investigate the use of ^{1}H MRS for this purpose.

Choline is essential for the normal function of cells, ensuring both structural integrity and signal functions of cell membranes and for influencing stem cell proliferation and apoptosis (3). The placenta delivers choline directly to the fetus but is also known to store large amounts of choline as acetylcholine (3). Lipids provide fuel for oxidative metabolism and are precursors for other compounds (4). They are delivered to the fetus in different ways e.g. via fatty acid binding proteins in the placenta (5). Glutamine and glutamate are important for fetal and placental nutrition and many other products are derived from them (6, 7). For example glutamine is involved in the synthesis of nucleotides and amino sugars required for the proliferation and differentiation of cells (6) and glutamate is a precursor of glutamine. The mother, placenta and the fetus are all involved in a delicate balance of the delivery and synthesis of glutamine and glutamate. The fetus receives glutamine from two sources, direct

transplacental transport from mother to fetus and glutamine synthesised within the placenta (7, 8). Glutamine is transported from the placenta to the fetus where it is used to synthesise glutamate in the fetal liver. The glutamate is then transported back to the placenta where it is converted back to glutamine (7). Day et al (8) suggest that placental glutamine and glutamate may also play a role in facilitating placental uptake of other amino acids and in passing waste products from the fetus to the mother. Therefore choline, lipids, glutamine and glutamate are known to be present in the placenta and their detection may have merit in the assessment of placental function.

Small for gestational age (SGA) fetuses (defined as a birthweight less than the 10^{th} centile) are at increased risk of perinatal morbidity and mortality (9). In SGA pregnancies, current obstetric management comprises fetal surveillance to try to predict fetal acidaemia thereby allowing timely delivery of SGA fetuses prior to irreversible end–organ damage and intrauterine fetal death. A wide range of tests including fetal biometry, biophysical profile (10), cardiotocograph (11), ultrasound assessment of amniotic fluid volume (12), and umbilical artery (13,14), middle cerebral artery (15), ductus venosus (16) and umbilical venous Doppler blood flow measurements (17) are used with varying efficacy. Despite reports in the literature indicating that placental function may be compromised in SGA pregnancies (18, 19), none of the currently available tests directly assess placental function *in utero*. Here ^{1}H MR spectra were acquired from the placenta of 9 SGA pregnancies and those of 9 healthy gestation matched subjects. The aim was to assess the ability of this technique to detect choline, lipids and the combined spectral contribution of glutamine and glutamate (Glx) in the placentae of these two groups.

Method

Ethics statement

The study was approved by Lothian Research Ethics Committee (11/SS/0031) and all participants gave written, informed consent.

Study population

Patients were enrolled at the Simpson Centre for Reproductive Health at the Royal Infirmary, Edinburgh, UK. Magnetic resonance studies were performed at the Clinical Research Imaging Centre in the Queen's Medical Research Institute, University of Edinburgh, UK.

9 women with a pregnancy complicated by SGA and 9 matched healthy controls were recruited prospectively. This is a pilot study and subjects were not derived from a larger cohort. Gestation was calculated from routine dating ultrasonography at 11-13 weeks gestation. To avoid bias, controls were prospectively recruited to match each recruited SGA pregnancy in turn for gestation (within 6 days) and smoking status. The inclusion criteria were a singleton pregnancy and >20 weeks gestation. The exclusion criteria were significant co-existing maternal systemic disease including microvascular disease, gestational diabetes, or contraindication to MRI. SGA was defined using ultrasound as an abdominal circumference $<10^{th}$ centile (20) in accordance with Royal College of Obstetricians and Gynaecologists guidelines (9, 21). SGA status was subsequently confirmed by a birthweight $< 10^{th}$ centile.

Doppler ultrasound scan

All participants had a transabdominal ultrasound scan performed using a Toshiba Aplio XG system (Toshiba Medical Systems Ltd, Crawley, UK) to assess fetal size, liquor volume and umbilical artery Doppler blood flow. Fetal size was assessed measuring head circumference

and abdominal circumference. Liquor volume was assessed subjectively with the amniotic fluid index being calculated if subjective assessment was abnormal. A free loop of the umbilical cord was interrogated with colour flow then pulsed Doppler in order to determine the resistance index. Centile charts for gestational age for Scottish singleton births were used to calculate birth weight percentiles (22).

^{1}H MRS Acquisition

All MR spectra were acquired using a wide-bore 3T MR Verio system (Siemens Healthcare Sector, Siemens AG, Erlangen, Germany). To avoid vena-cava compression, women were placed in a left-lateral tilt, with blood pressure being constantly monitored using a Veris MR Vital Signs Monitor (Medrad, UK). No fetal sedation was used and each participant was limited to spending 45 minutes in the scanner. Placenta location and orientation was confirmed using standard half-Fourier rapid MRI acquisitions and used to spatially select the MRS voxel. No other structural MR scans were acquired. A single voxel point resolved spectroscopy (PRESS) technique was applied with TR/TE =1500ms/30ms, 96 signal averages, bandwidth of 2000Hz and a water suppression bandwidth of 50Hz. Each spectral acquisition took 2 minutes 30 seconds. Signal was received from selected elements of the spine matrix coil and body matrix surface coils positioned to allow adequate coverage of placenta. Following the acquisition of scout images, the scanner bed was moved to ensure that placenta was positioned at isocentre and great care was taken to position the $20\times20\times40mm^3$ voxel within the placenta and to avoid any contaminant signal from surrounding tissue. An example of voxel positioning for MRS acquisition is shown in Figure 1. An optimised semi-automated shimming protocol was systematically applied until the full width at half maximum of the water peak was <30Hz. Significant movement of the placenta is not expected during spectral acquisition and data were acquired with the mother free breathing throughout.

Spectral analysis

All spectral analysis was carried out using jMRUI (23). Water peak amplitudes were first estimated for each spectrum using the advanced method for accurate, robust and efficient spectral fitting (AMARES) a semi-automatic quantification method (24). Analysis of overlapping *in utero* placenta spectral components was then carried out using the Quest algorithm (23, 25). The Quest algorithm is a time domain spectral analysis tool which estimates metabolite amplitudes using a non-linear least squares fit of a weighted combination of simulated metabolite signals to the acquired spectrum. As recommended in the literature (25), the intrinsic water peak was removed using the Hankel Lanczos Singular Values Decomposition Filter (HLSVD) prior to metabolite quantification. For this purpose a metabolite basis set was generated using the NMR-Scope function also available in jMRUI (25) and included contributions from the overlapping resonances of glutamine and glutamate (Glx) between 2.06-2.44ppm and between 3.76-3.78ppm, from choline (3.2, 3.53 and 4.08ppm) and fatty acids (1.3 and 0.9ppm). Separate simulations of glutamine and glutamate were generated and then summed using the jMRUI pre-processing tool to create a simulation of Glx. The Quest algorithm calculates errors associated with the estimated metabolite amplitudes using an extended version of the Cramer-Rao lower bounds calculation (25). The errors for each of the calculated metabolite ratios were derived through error propagation of this jMRUI output. The Quest algorithm carries out a reproducible estimation and subtraction of the baseline and has previously been subject to extensive Monte Carlo studies that have validated its reliability with respect to bias and variance (25).

An example of the Quest algorithm output for SGA subject 5 is shown in Figure 2. This shows the acquired spectrum (Figure 2a), the estimated spectrum (Figure 2b), the simulations of individual metabolites (Figure 2c) and the residue function (Figure 2d) following fitting of

the estimated spectrum to the acquired spectrum. Glx, choline and both lipid peaks at 1.3ppm and 0.9ppm were quantified in terms of ratios to the amplitude of the intrinsic water peak (i.e. Glx/H_2O, choline/H_2O, Lipid 1.3ppm/H_2O and Lipid 0.9ppm/H_2O).

Statistical tests

The null hypotheses are that no differences exist between the following metabolite ratios of matched SGA and control placentae: 1) Glx/H_2O; 2) choline/H_2O; 3) Lipid contribution at 1.3ppm (Lipid 1.3ppm/H_2O); and 4) Lipid contribution at 0.9ppm (Lipid 0.9ppm/H_2O). Not all metabolite ratio differences conformed to a normal distribution. Therefore separate Wilcoxon Signed Rank tests were carried out on paired differences of each of the four metabolite amplitude ratios using Minitab ® 16 Statistical Software (Minitab, Inc. www.minitab.com). Significance was defined as $p<0.05$.

Results

Demographics of study population

Mean maternal age range was 28.5 ± 7.6 years and mean maternal body mass index (BMI) was 23.3 ± 6.0 kg/m^2. The maternal demographics and the population characteristics at delivery are shown in Table 1.

Perinatal outcome

Perinatal outcomes are demonstrated in Table 2. All SGA subjects were confirmed by a birthweight of <10^{th} centile. Five out of the nine SGA subjects delivered preterm (defined as <37 weeks gestation), whereas all controls delivered at term. All controls and seven out of nine SGA subjects were discharged home alive and well. One SGA subject was stillborn and one had a neonatal death following surgery for gastroschisis. Three SGA subjects were

admitted to the neonatal unit (NNU), two following surgery for gastroschisis and one due to low temperature and hypoglycaemia secondary to low birthweight. Two controls were admitted to the NNU, one due to suspected sepsis and the other following an unplanned home delivery. Other than the two SGA subjects with gastroschisis, all other controls and subjects had no genetic or structural abnormalities.

^{1}H MRS

In utero ^{1}H MRS of the placenta were obtained in all 9 SGA pregnancies and in all 9 healthy control subjects. The spectrum acquired from control 6 is shown in Figure 3. In Figure 3a the water peak is dominant at 4.7ppm and in Figure 3b the water peak has been filtered out to reveal the smaller amplitude metabolite peaks. Spectral peaks corresponding to choline, Glx and lipids are indicated. The amplitude ratios of choline, Glx and the lipid peaks at 1.3ppm and 0.9ppm to the intrinsic water peak at 4.7ppm (i.e. Choline/H_2O, Glx/H_2O, Lipid 1.3ppm/H_2O, Lipid 0.9ppm/H_2O) are shown in Table 3.

No significant difference in paired SGA-control placental choline/H_2O ratios was found ($p=0.722$). This suggests that placental choline metabolism is preserved in this SGA cohort. A significant difference ($p=0.024$) was found between paired SGA-control placental Glx/H_2O ratios, suggesting that Glx metabolism is altered in this cohort. Finally the amplitude ratios of Lipid 1.3ppm/H_2O and Lipid 0.9ppm/H_2O did not demonstrate a significant difference between matched SGA-control pairs ($p=0.813$ and $p=0.058$ respectively).

Discussion

To our knowledge, this is the first *in utero* placenta ^{1}H MRS study to assess choline and lipid using the internal water peak as an independent reference and to additionally assess placental glutamine and glutamate as it supports the developing fetus. This study has shown that the

Glx/H_2O ratios of the SGA placentae and gestation-matched healthy control placentae were significantly different but that their choline/H_2O and lipid/H_2O ratios were not. This suggests that in this cohort of SGA placentae, metabolism of Glx is altered but that choline and lipid metabolism is preserved.

The finding that placental Glx is significantly reduced in a cohort of 9 SGA subjects is consistent with other studies in the literature. Firstly there are general reports of a reduction in most essential amino acid concentrations in the SGA fetus prior to delivery (26, 27). There are also reports of a reduction in the amino acid transport mechanisms of the placenta in SGA pregnancies (18, 19). More specifically, in an ovine fetal growth restricted model Regnault et al (28) found that there was a significant reduction in the umbilical uptake of glutamine and in the placental uptake of fetal glutamate. Hill et al (29) identified 3 different transport mechanisms *in vitro* that allow glutamine uptake across the microvillous plasma membrane (MVM) of the placenta. They report that the majority of NA^+-dependent and NA^+-independent transport of glutamine across the MVM was mediated by mechanisms that are known to be reduced in fetal growth restriction (FGR) pregnancies, a severely compromised subset of SGA pregnancies.

As previously described (7), the mother, placenta and the fetus all form part of a delicate glutamine-glutamate shuttle. Glutamine is both transported across the placenta from the mother and is also manufactured in the placenta (6-8), using glutamate which is synthesised in the fetal liver. Therefore any disruption in the supply or transport mechanism of either amino acid from any one source could potentially disrupt this fine balance of placental Glx metabolism. It is known from studies in the porcine placenta (6) that both glutamine and glutamate vary with gestational age. However, by limiting analysis to comparisons of SGA-control pairs matched to gestational age, the influence of gestation on glutamine and glutamate is removed.

The Glx/H_2O ratio was found to be reduced in 7 of the SGA subjects (Table 3) compared to their matched control but was increased in 2 subjects (SGA 4 and 7) compared to their matched controls. These subjects also had reversed end diastolic flow (REDF) and raised flow in their umbilical artery respectively. It is beyond the scope of this article to make conclusions regarding clinical outcomes but it should be noted that SGA subject 4 was stillborn 10 days after MRS acquisition. Therefore it is likely that placental metabolism was significantly altered in this case. Regnault et al (28) report that even in the most severe SGA cases where FGR is confirmed, the fetus is not consistently in a state of constant hypoxia and so may have access to higher concentrations of amino acids at different times. Their work indicates that in severe SGA with FGR there is a reduction in placental O_2 transport which reduces fetal metabolism. They suggest that this reduction in metabolic rate may be greater than the reduction in placental amino transport capacity, leading to normal or higher than normal amino acid concentrations. It is possible that this mechanism may account for the relative increase in Glx/H_2O ratios in SGA subjects 4 and 7 compared to their respective control subjects.

This study found that there were no significant differences in choline/H_2O ratios between SGA placentae and matched control placentae. Choline is vital for normal brain development, including the prevention of neural tube defects (3). Choline passes from mother to fetus via the placenta against a concentration gradient and both fetal tissue and amniotic fluid have very high choline concentrations compared to maternal levels (3). Despite this there is evidence that reproductive age women are relatively resistant to choline deficiency due to an oestrogen driven process to produce phosphatidylcholine from precursors in the liver (3, 30). The importance of choline to fetal neurodevelopment and the different reserves that the mother can use to supply choline to the fetus could perhaps explain the preserved choline levels in SGA placentae compared to controls found in this study.

Individual SGA subjects 4, 5 and 7 had increased choline compared to their matched control subject. As discussed above SGA subjects 4 and 7 had REDF and raised flow in the umbilical artery respectively. Subject 5 also had raised umbilical artery flow. This anecdotal finding is consistent with previous larger studies (31, 32) that have measured choline concentrations in venous umbilical cord blood samples in SGA and normal pregnancies. Venous blood samples reflect maternal choline concentrations, maternal-placental choline transfer and placental choline metabolism as opposed to arterial blood samples that reflect fetal metabolism (31). Although both studies hypothesised that a status of SGA and subsequent low birth weight would result in low choline levels, in fact they observed high choline concentrations in the umbilical cord blood samples of these neonates. Hogeveen et al (31) suggested two reasons for this. The first is that high choline concentrations reflect low choline consumption by the fetus and the second is the potential that slow fetal growth triggers an increased placental transport of choline.

Assessment of the Lipid 1.3ppm/H_2O ratios, the dominant lipid peak, of individual SGA-control pairs showed that there was an increase for SGA subjects 5, 7 and 9 compared to their control subject. These subjects all had raised umbilical artery Doppler ultrasound measurements. Interestingly 2 of these SGA subjects also had increased placental choline/H_2O ratios. Choline is a precursor for the phospholipid phosphatidylcholine, a main constituent of membranes, lipoproteins, bile and surfactants (33). Therefore it is possible that placental choline and lipid metabolism are related.

A previous study (34) demonstrated a reduction in the placental choline/lipid ratio using *in vivo* ^{1}H MRS, acquired with TE 144ms, in 3 pregnancies complicated by severe SGA with FGR. All subjects in that study in that study had reduced liquor volume and absent end diastolic flow in the umbilical artery. That study speculated that this may be a biomarker of critical placental failure indicated by reduced cell turnover. These subjects were recruited as

their placental function was known to be severely compromised and were confirmed to be suffering from placental mediated FGR. Conversely the present study concerns pregnancies not confirmed to be suffering from FGR. Spectra were acquired using a lower TE of 30ms allowing the detection of Glx in addition to choline and lipid. Significant differences were not detected between the choline/lipid 1.3ppm ratios (Wilcoxon signed rank test p= 0.286, level of significance $p<0.05$) or the choline/lipid 0.9ppm ratios ($p=0.155$) of SGA placentae and those of matched controls (data not shown). This was expected following confirmation that no significant differences were observed in choline/H_2O ratios or either of the lipid/H_2O ratios between these groups. It was also expected that as these two studies considered subjects with different clinical characteristics, their choline and lipid metabolisms would be different. In the future it would be of interest to assess placental Glx/H_2O ratios in known cases of severe FGR.

Mckelvey and Kay (35) carried out a review of MRS studies of the placenta. Of these studies only 2 applied ^{1}H MRS to study *ex vivo* placental tissue samples. Serkova et al (36) calculated absolute placental concentrations of major cellular metabolites including glutamine, glutamate, phosphocholine, glycerophosphocholine and lipids in extracts of normal placenta collected at different time points following delivery. Pulkkinen et al (37) found that normal *ex vivo* placenta samples had higher phospholipid content than normal myometrium tissue samples. The only *in vivo* study reported was by Weindling et al (38) who acquired ^{31}P MR spectra on a 1.5T scanner from an anteriorly placed placenta in 7 women with normal pregnancies. Therefore there is currently no opportunity to directly compare the results from the present study to any other published data.

We accept that the acquired *in utero* placenta spectra may contain overlapping contributions from different metabolites. Therefore it is to be expected that spectral resolution will be lower than is possible in *ex vivo* studies. Previous *ex vivo* and *in vivo* studies have attributed

metabolite peaks detected at ~ 2 parts per million (ppm) to Glx (39, 40). However one possible contaminant may simply be polyunsaturated fatty acids (41). In addition Takeuchi et al (42) suggested that a peak detected at ~2ppm in a ^{1}H MRS study of ovarian tumours could be attributed to N-acetyl mucinous compounds (i.e. those containing the glycosylated protein mucin). There is evidence in the literature that mucin-15 is expressed in the human placenta to varying degrees at different stages of gestation (43), but equally there is evidence that glutamine is present in ovarian tumours (44). Takeuchi et al (42) concede that their results also suggest contamination from other sources. Using a powerful spectral analysis tool such as the Quest algorithm is imperative to allow contributions form overlapping spectral metabolites to be quantified.

This study is limited by its small sample size and heterogeneity in demographics and clinical characteristics of study participants. There is no evidence that factors such as maternal age, BMI and parity would influence the results. All but one of the control subjects had an abdominal circumference above the 50^{th} centile. Although control subjects were not selectively recruited to have abdominal circumferences above the 50^{th} centile, this may potentially influence observed placental metabolite differences between SGA and control subjects. Two of the fetuses had gastroschisis. There is evidence that placental dysfunction is an important causative factor in cases of SGA with gastroschisis (45). However, we accept that other factors will also play a role in causing SGA in fetuses with gastroschisis and that this may be a potential limitation in the SGA group. Four SGA subjects had abnormal umbilical artery flow measurements. It is known that umbilical artery Doppler ultrasound measurements are often normal in the SGA fetus (46, 47). This study did not aim to and was not powered to detect differences in placental metabolites in SGA pregnancies with and without evidence of abnormal Doppler measurements. However in future studies it would be preferable to sub-classify subjects into groups of normal and abnormal Doppler

measurements. The application of ^{1}H MRS to study the *in utero* placenta requires specialist knowledge and so may not be immediately transferrable to all centres or all patients. Future studies would benefit from acquiring placental volume scans which could then be assessed for correlation with metabolite ratios.

In conclusion, this study suggests that ^{1}H MRS can be used to detect placental metabolites *in utero*. We have demonstrated an ability to detect significant differences in Glx in the placentae of SGA pregnancies *in utero* and can demonstrate maintained choline and lipid levels in these pregnancies. Although these findings cannot currently be used to inform clinical decisions, these encouraging initial results indicate that ^{1}H MRS warrants further investigation in the assessment of *in utero* placental function. This is a new application of an established technique and in the first instance further tests in larger populations are required to confirm these results.

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List of Tables and Figures

the subsequent spectral analysis of other metabolites. Spectral contributions from Glx, choline and lipid are indicated.

Table 1: Maternal demographics

Subject	Gestational age at 1H MRS (weeks $^{+days}$)	Smoker (Y/N)	Age	Parity	BMI	Abdominal circumference ultrasound centile	Umbilical artery flow	Liquor volume
SGA 1	33^{+3}	N	36	1+0	19	5^{th}	Normal	Reduced
Healthy 1	33^{+4}	N	33	0+0	24.2	25^{th} -50^{th}	Normal	Normal
SGA 2	33^{+2}	Y	32	1+0	18.3	$<5^{th}$	Normal	Normal
Healthy 2	33^{+5}	Y	18	0+0	18.7	50^{th} – 95^{th}	Normal	Normal
SGA 3	29^{+2}	Y	26	2+3	23.6	5^{th}	Normal	Reduced
Healthy 3	29^{+3}	Y	20	0+0	19.5	75^{th}	Raised	Normal
SGA 4	26^{+0}	N	41	0+0	39.7	$<5^{th}$	REDF	Normal
Healthy 4	26^{+6}	N	24	1+1	30.5	75^{th}	Normal	Normal
SGA 5	36^{+6}	N	26	1+1	23.1	5^{th}	Raised	Normal
Healthy 5	36^{+1}	N	38	0+2	18.8	95^{th}	Normal	Normal
SGA 6	31^{+4}	N	27	0+0	19.8	5^{th} – 10^{th}	Normal	Normal
Healthy 6	32^{+0}	N	39	2+0	20.9	95^{th}	Normal	Normal
SGA 7	37^{+3}	Y	28	2+1	30.2	$<5^{th}$	Raised	Normal
Healthy 7	38^{+2}	Y	22	2+0	23.7	25^{th}	Normal	Normal
SGA 8	33^{+5}	N	18	0+0	19.2	$<5^{th}$	Normal	Normal
Healthy 8	33^{+3}	N	28	1+0	20.7	95^{th}	Normal	Normal
SGA 9	28^{+5}	N	33	0+0	23.0	5^{th}-10^{th}	Raised	Normal
Healthy 9	29^{+2}	N	35	1+1	20.3	95^{th}	Normal	Normal

Table 2: Perinatal outcome. Abbreviations: HFFD Haag Ferguson Forceps Delivery; SVD Spontaneous Vertex Delivery; EmCS Emergency Caesarean Section; NNU Neonatal Unit; RDS Respiratory Distress.

Subject	Time between MRI and delivery (days)	Gestational age at delivery (weeks^{+days})	Mode delivery	Sex	Birthweight (g)	Percentile at birth	Outcome
SGA 1	46	40^{+1}	SVD	F	2610	$<2^{nd}$	Alive and well
Healthy 1	44	39^{+6}	HFFD	M	2960	9^{th} - 25^{th}	NNU admission for 5 days with suspected sepsis. Alive and well
SGA 2	23	36^{+4}	SVD	M	1610	$<2^{nd}$	Gastroschisis, surgery, neonatal death 11 days of age
Healthy 2	45	40^{+1}	SVD	M	3420	25^{th} -50^{th}	NNU admission for 2 days with RDS following unplanned home delivery. Alive and well
SGA 3	44	35^{+4}	EmCS	F	1690	2^{nd}	Gastroschisis surgery, alive and well.
Healthy 3	83	41^{+1}	SVD	F	3640	50^{th} – 75^{th}	Alive and well
SGA 4	10	27^{+3}	EmCS	F	500	$<2^{nd}$	Stillbirth 10 days after MRI scan
Healthy 4	102	40^{+3}	SVD	F	3650	50^{th} -75^{th}	Alive and well
SGA 5	2	37^{+0}	EmCS	F	1840	$<2^{nd}$	Alive and well
Healthy 5	24	39^{+4}	SVD	M	4020	91^{st}	Alive and well
SGA 6	40	37^{+2}	EmCS	F	1710	$<2^{nd}$	NNU admission for 5 days with low temperature and hypoglycaemia. Alive and well
Healthy 6	53	39^{+4}	SVD	F	3670	75^{th} – 91^{st}	Alive and well
SGA 7	4	38^{+1}	EmCS	F	1980	$<2^{nd}$	Alive and well
Healthy 7	9	39^{+4}	SVD	M	3170	25^{th}	Alive and well
SGA 8	3	34^{+1}	ElCS	M	1670	$<9^{th}$	Alive and well
Healthy 8	56	41^{+3}	SVD	F	3500	25^{th} - 50^{th}	Alive and well
SGA 9	42	34^{+5}	EmCS	M	1570	$<2^{nd}$	Admitted to NNU, RDS for 21 days. Alive and well
Healthy 9	81	39^{+6}	SVD	M	3890	75-91^{st}	Alive and well

Table 3: Placenta amplitude ratios of Glx/H_2O, Cho/H_2O and Lipid 1.3ppm/H_2O for SGA and gestation matched healthy controls quantified from placenta ^1H MR spectra using jMRUI. Standard deviations, derived through error propagation of those estimated by jMRUI, are also quoted. H_2O refers to the intrinsic water peak of each individual spectrum observed at 4.7ppm. N/A indicates that the metabolite was not detected and * indicates that the amplitude ratio for the SGA placenta is higher than that of the matched control. Wilcoxon signed rank test p values (significance < 0.05) are quoted for SGA-healthy subject comparisons for each ratio.

Metabolite amplitude ratios ± stdev				
	Glx/H_2O ($\times10^{-3}$)		**Choline/H_2O ($\times10^{-3}$)**	
Matched Pairs	**SGA**	**Healthy**	**SGA**	**Healthy**
SGA 1 /Healthy 1	1.42 ± 0.02	2.85 ± 0.04	0.50 ± 0.01	0.80 ± 0.08
SGA 2 /Healthy 2	0.68 ± 0.02	1.83 ± 0.05	0.18 ± 0.05	0.31 ± 0.13
SGA 3 /Healthy 3	0.47 ± 0.01	3.66 ± 0.04	0.52 ± 0.01	0.69 ± 0.10
SGA 4 /Healthy 4	2.67 ± 0.03*	2.38 ± 0.06	0.63 ± 0.02*	0.36 ± 0.10
SGA 5 /Healthy 5	6.48 ± 0.07	9.76 ± 0.10	1.36 ± 0.17*	0.81 ± 0.18
SGA 6 /Healthy 6	0.89 ± 0.03	3.08 ± 0.06	0.53 ± 0.11	0.83 ± 0.04
SGA 7 /Healthy 7	2.44 ± 0.09*	1.38 ± 0.03	0.83 ± 0.14*	0.38 ± 0.08
SGA 8 /Healthy 8	0.28 ± 0.08	2.12 ± 0.04	0.61 ± 0.22	0.74 ± 0.03
SGA 9 /Healthy 9	4.00 ± 0.08	5.39 ± 0.06	1.07 ± 0.22	0.90 ± 0.03
p-value	**0.024**		**0.722**	
	Lipid 1.3ppm/H_2O ($\times10^{-3}$)		**Lipid 0.9ppm/H_2O ($\times10^{-3}$)**	
Matched Pairs	**SGA**	**Healthy**	**SGA**	**Healthy**
SGA 1 /Healthy 1	3.27 ± 0.41	7.59 ± 0.53	3.84 ± 0.10	5.43 ± 0.25
SGA 2 /Healthy 2	0.96 ± 0.27	8.17 ± 0.59	0.44 ± 0.08	5.99 ± 0.23
SGA 3 /Healthy 3	3.50 ± 0.37	8.51 ± 0.68	3.87 ± 0.08	5.08 ± 0.20
SGA 4 /Healthy 4	0.68 ± 0.39	2.78 ± 0.63	2.52 ± 0.15	2.61 ± 0.88
SGA 5 /Healthy 5	44.9 ± 1.35*	16.5 ± 1.34	11.9 ± 0.37	36.6 ± 1.37
SGA 6 /Healthy 6	5.69 ± 0.74	15.1 ± 0.91	5.63 ± 0.19	7.69 ± 0.22
SGA 7 /Healthy 7	43.8 ± 1.46*	6.83 ± 0.40	6.70 ± 0.48*	2.54 ± 0.13
SGA 8 /Healthy 8	4.62 ± 1.58	4.32 ± 0.62	N/A	3.33 ± 0.20
SGA 9 /Healthy 9	23.1 ± 1.82*	8.72 ± 0.87	2.94 ± 0.34	9.83 ± 0.29
p-value	**0.813**		**0.058**	